In-vitro anticoagulant activity of three extracts of Astragalussarcocolla

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ABSTRACT

Aim: To access the anticoagulant effect of n-hexane (HE), chloroform (CE) and distilled water (DE) extracts of A. sarcocolla.

Methodology: Resin of A. sarcocolla was extracted using n-hexane, chloroform and distilled water using maceration. The extract was concentrated using rotary evaporator. Four different doses of extract (2.5, 5, 7.5 and 10 µg) were prepared for performing assay. In-vitro anticoagulant effects using human blood were studied in 42 healthy males age between 25-35 years.

Results: All extracts significantly increased the PT in comparison to control. At highest dose of 10µg, with the exception of distilled water extract, all extracts demonstrated highly significant (p<0.001) increase in APTT.

Conclusion: The data of the present study demonstrates that extracts of A. sarcocolla possess anticoagulant activity.

Keywords: Anticoagulant; Astragalussarcocolla; Prothrombin time; Activated Partial Thromboplastin time

INTRODUCTION

Coagulation is the process that involves fibrin formation through controlled interaction between protein coagulation factors1. Hyper-coagulation must be kept in check because it has been associated with cardiovascular diseases. A large number of plants have been found as the rich source of anticoagulant constituents therefore, researchers have been continuously looking for discovering such constituent’s world over which have high efficacy with minimal side effects2. For this purpose, screening of A. sarcocolla was done to study its effect on blood coagulation. Activated partial thromboplastin time (APTT) and prothrombin time (PT) are the most commonly used tests to assess the integrity of coagulation system. APTT measures the intrinsic pathway while PT assesses the functioning of extrinsic pathway.

Astragalus L., is one of the biggest genuses of flowering plants in the leguminasae family. Astragalus species have been used in folk medicine as anti-thrombotic, anti-inflammatory, immunostimulant, antioxidative, antineoplastic, antidiabetic, cardioprotective, hepatoprotective and antiviral agents3. Numerous researchers have documented that plants with anti-inflammatory properties can inhibit coagulation4,5. The study was designed on hypothesis based on folk uses and few articles claiming that that genus "Astragalus" do have anti-inflammatory activities6,7. A. sarcocolla has not so far been studied for its anticoagulant potential. The objective of this study is to access whether A. sarcocolla possesses anticoagulant activities in terms of PT and APTT.

MATERIALS AND METHODS

The A. sarcocollaplanetresin was obtained from local herbarium market in September 2019, Lahore, Pakistan. Plant was grinded down to powder form and maceration was carried out according to the method described by Williamson6,9. The powdered resin of plant was sequentially extracted (1:5) with different solvents according to their polarity in the increasing order that is, n-hexane (ASHE), chloroform (ASCE) and distilled water (ASDE) for 72 hours at room temperature with occasional manual stirring. The macerated plant material was filtered through muslin cloth and vacuum filtered through Buchner funnel using Whatman filter paper no.1. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator N-1000 at the temperature of 40°C10 for 1 hour then oven dried until it was turned into paste. Dried extract was collected and stored in air tight container in a refrigerator until use. This dried extract was used in this current experiment. Four different doses of extract (2.5, 5, 7.5 and 10 µg) were prepared for performing assay. ASHE and ASCE were dissolved using 1% carboxymethyl cellulose (CMC) while normal saline was used to dissolve ASDE.

The study protocol was approved by the Ethical Committee of Riphah Institute of Pharmaceutical Sciences (RIPS), Lahore, Pakistan. The anti-platelet aggregation activity of crude plant extract material on human platelets was assessed by the procedure described by Shah11.

Venous blood was drawn from antecubital vein from healthy human volunteers (N=42) free of medication for at least 14 days. 9 ml blood was transferred into the 15 ml conical tube containing anticoagulant 1 ml 3.8% (w/v) trisodium citrate solution at the ratio of 9:1. Citrated blood samples were centrifuged at 4000 rpm for 10 min using Compact Centrifuge Z 206 A (HERMLE Labortecnik GmbH Siemens, Germany) to obtain platelet poor plasma (PPP)11.
Plasma coagulation assays were performed using Sysmex coagulation analyzer CA-1500 (Sysmex Corporation Kobe, Japan). Determination of PT and APTT was carried out in accordance with the manufacturer’s recommended protocols (Siemens). The PPP was freshly prepared as mentioned in the preceding paragraph. Plasma mixtures were prepared by mixing 100 μL of plasma with 50 μL of test sample before performing the PT, and APTT assays12. Negative Control consisted of normal saline or 1% CMC instead of extract.

**Prothrombin Time (PT):** After incubation at 37°C for 180 seconds (s), 100 μL of the plasma mixture was blended with 200 μL of Thromborel® S. The reaction was monitored for 120 s.

**Activated Partial Thromboplastin time (APTT):** After incubation at 37°C for 60 s, 100 μL of the plasma mixture was mixed with 100 μL Dade Actin® FSL and incubated for another 180 s. Then, 100 μL of calcium chloride was added to the reaction mixture and monitored for 190 s.

**Statistical Analysis:** Statistical analysis was performed by using GraphPad Prism 5 (Graph Pad Software, La Jolla, CA, USA). The study variables in six groups were compared using two-way ANOVA and differences among groups was determined by Bonferroni’s post hoc test. All experimental values reflect an average of a minimum of 3 experiments (mean ± SEM). The p-values <0.05 was considered statistically significant.

**RESULTS**

As the dose was increased PT was also increased. All extracts significantly increased the PT in comparison to control. ASHE showed comparable results with control. ASCE at 10μg, displayed most significant results among all (29.61 ± 2.27 sec). Figure 1 (a)

At dose of 2.5μg, only ASCE displayed highly significant (p<0.001) increase in APTT. No increase in APTT was observed with the remaining extracts. At 5μg ASCE displayed significant increase in APTT when compared with control. No increase in APTT was observed with ASDE. At dose of 7.5μg, most significant (p<0.001) increase in APTT was shown by ASCE. ASDE failed to increase APTT.

At highest dose of 10μg, with the exception of ASDE, all extracts demonstrated highly significant (p<0.001) increase in APTT. Figure 1 (b).

Figure 1: Effect of various extracts of *A. sarcocolla* in different concentrations on *in-vitro* coagulation profile (a. PT, b. APTT) against control using Post Hoc Bonferroni’s test (Compared with control, *P<0.05, **P<0.01, ***P<0.001, n = 6, mean ± SEM

![Graph showing effect of various extracts on PT and APTT](image-url)
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DISCUSSION

A recent survey has pointed out that subcontinent region of the world is at high risk of morbidity and mortality due to cardiovascular diseases (CVD). In 2014, WHO published a report stating that deaths in Pakistan due to CHD have reached 9.87% of total number of deaths. 30-40 % of all deaths in Pakistan are due to CVD. Anticoagulant drugs are commonly used to treat thromboembolic conditions. Drug interactions and fatal side effects are the reasons due to which researchers throughout the world have been searching for the natural compounds with highest efficacy and fewer side effects.

Astragalus genus is rich in multiple constituents which are known to have anticoagulant activities. Multiple biological activities like antioxidant, antiplatelet, anticoagulant and antithrombotic are attributed to polysaccharides. Various researchers have demonstrated the anticoagulant activity of tannins. Some terpenoids are recognized to display antioxidant activities. Sapnoins are known to possess antiplatelet, anticoagulant as well as fibrinolytic characteristics.

PT is used to evaluate the integrity of extrinsic pathway of blood coagulation. ASHE showed no increase in PT even at a higher dose. ASCE displayed significant increase in PT as compared to the control at higher doses of 7.5 and 10μg. Surprisingly ASDE also displayed significant increase in PT at all concentrations.

APPT is used to assess the integrity of intrinsic pathway of blood coagulation as it monitors factors XII, XI, IX, VIII, V, II, I, prekallikrein, and high molecular weight kininogen. ASCE displayed dose dependent increase in APTT. Increase in APTT by ASCE was strongest among all extracts used in this study. ASHE produced significant increase in APTT as compared to the control only at 10μg. No increase in APTT was observed with ASDE indicating that its components do not affect the intrinsic pathway of blood coagulation.

As FXa is critical for the generation of thrombin from prothrombin during propagation phase of coagulation cascade. Thus, one of the simplest explanations of prolonged PT could be blockage of FXa or formation of prothrombinase complex. Findings from PT and APTT suggest that A. sarcocolla has those constituents that effect the blood coagulation pathway either through inhibition of clotting factors involved in intrinsic pathway or by enhancing the activity of AT.
CONCLUSION

The results of the present study have revealed that A. sarcocolla possesses anticoagulation potential. ASCE showed significant anticoagulant activity at higher concentrations. This study provides a ground for future research on A. sarcocolla to isolate most active constituents responsible for the anticoagulant ability that can pave the way to make a pure drug which could combat CVD. Detailed in vivo studies are required before any conclusion regarding the medicinal role and usefulness of A. sarcocolla.

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